Assessment of a $\delta^{15}N$ Isotopic Method to Indicate Anthropogenic Eutrophication in Aquatic Ecosystems

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ABSTRACT

Increased anthropogenic delivery of nutrients to water bodies, both freshwater and estuarine, has caused detrimental changes in habitat, food web structure, and nutrient cycling. Nitrogen-stable isotopes may be suitable indicators of such increased nutrient delivery. In this study, we looked at the differences in response of macrophyte $\delta^{15}N$ values to anthropogenic N across different taxonomic groups and geographic regions to test a stable isotopic method for detecting anthropogenic impacts. Macrophyte $\delta^{15}N$ values increased with wastewater input and water-column dissolved inorganic nitrogen (DIN) concentration. When macrophytes were divided into macroalgae and plants, they responded similarly to increases in wastewater N, although macroalgae was a more reliable indicator of both wastewater inputs and water-column DIN concentrations. Smooth cordgrass (*Spartina alterniflora* Loisel.) $\delta^{15}N$ increased uniformly with wastewater inputs across a geographic range. We used the relationship derived between *S. alterniflora* and relative wastewater load to predict wastewater loads in locations lacking quantitative land use data. The predictions matched well with known qualitative information, proving the use of a stable isotopic method for predicting wastewater input.

INCREASES IN HUMAN POPULATION in coastal watersheds have increased delivery of nutrients to lakes, ponds, and estuaries (Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection, 1990; National Research Council, 1994). The resulting eutrophication has many adverse effects within the estuaries (Duarte, 1995; D'Avanzo et al., 1996; Hauxwell et al., 1998). Increased N loading can lead to blooms of phytoplankton and macroalgae (Duarte, 1995; Hauxwell et al., 1998). These blooms in turn lead to the loss of important estuarine habitats like seagrass meadows. The loss of seagrass meadows is accompanied by the loss of important commercial shellfish and finfish species such as cod (Tveite, 1984), bay scallops (Pohle et al., 1991), and blue crabs (Heck and Orth, 1980). Eutrophic estuaries can also suffer from anoxia (Zimmerman and Canuel, 2000), harmful algal blooms, and brown tides (Hodgkiss and Ho, 1997).

These adverse effects have prompted search for suitable indicators of eutrophication to assess water quality of aquatic ecosystems. Nitrogen-stable isotopes have been suggested as such indicators (Cabana and Rasmussen, 1996; McClelland et al., 1997; McClelland and Valiela, 1998; Lake et al., 2001; McKinney et al., 2001; Wigand et al., 2001; Cole, unpublished data, 2002) for freshwater and estuarine systems.

Differences in ratios of ^{15}N to ^{14}N have been used to define food webs, as well as natural tracers of N sources (Fry and Sherr, 1984; Peterson and Fry, 1987). The ratio of ^{15}N to ^{14}N is expressed as $\delta^{15}N$ (‰) $= [(R_{sample} - R_{reference})/R_{reference}] \times 1000$, where R is $^{15}N/^{14}N$ and the reference is atmospheric N_2 (Peterson and Fry, 1987). Wastewater N in ground water typically has a $\delta^{15}N$ of +10 to +22‰, largely because of denitrification and volatilization of ammonia in septic system leaching fields (Kreitler et al., 1978; Kreitler and Browning, 1983; Aravena et al., 1993; Macko and Ostrom, 1994). This range is significantly higher than the $\delta^{15}N$ of ground water N derived from atmospheric deposition (+2 to +8‰; Kreitler et al., 1978; Kreitler and Browning, 1983), and from fertilizer (−3 to +3‰, Kreitler et al., 1978; Kreitler and Browning, 1983).

The $\delta^{15}N$ values in primary producers, macrophytes and phytoplankton, reliably reflect N inputs from land to water bodies (McClelland et al., 1997; Voss and Struck, 1997; McClelland and Valiela, 1998; Waldron et al., 2001; Cole, unpublished data, 2002), and also are significantly related to DIN concentrations in the receiving waters (Cole, unpublished data, 2002). Although, $\delta^{15}N$ of primary producers is more clearly correlated to the percentage wastewater contribution than to N loads (Cole, unpublished data, 2002).

The $\delta^{15}N$ of primary producers may vary because of differences in taxonomy and geography. Differences in the geographic location of water bodies introduce differences in species composition, climate, and water and sediment characteristics; many of these features could affect the $\delta^{15}N$ of producers (Peterson and Fry, 1987). Plants acquire N from the sediment, but algae N uptake occurs through fronds (Duarte, 1995). This taxonomic-based contrast may create a different $\delta^{15}N$ in each producer type since N in sediment and the water column often differ in $\delta^{15}N$. The $\delta^{15}N$ of producers in freshwater and estuaries may also differ because of the marked differences between freshwater and estuarine environments in N supply and in N transformations (Valiela, 1995).

In this report, as a first objective we first test the $\delta^{15}N$

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Abbreviations: ANCOVA, analysis of covariance; DIN, dissolved inorganic nitrogen; NLM, nitrogen-loading model; POM, particulate organic matter.

approach by applying it to a geographically broad range of water bodies to assess how well the N-stable isotopic content of plants, macroalgae, and particulate organic matter (POM) are correlated to two indicators of anthropogenic eutrophication, water-column DIN concentrations, and percentage of land-derived N load that is contributed by wastewater. We use both new and previously collected data to compare producer $\delta^{15}N$ to percentage wastewater inputs, and to water-column DIN concentrations in fresh and salt water bodies in the U.S. East and West Coasts, and in Brazil. As a second objective, we further extend the use of $\delta^{15}N$ in macrophytes to predict wastewater inputs to aquatic systems where we lack quantitative information on N or wastewater inputs from land.

METHODS

We assessed how the relationship between macrophyte $\delta^{15}N$ and relative wastewater load changed with different species and with different geographical areas. We first determined how macroalgae and plants responded to wastewater inputs and to water-column DIN concentrations. We then compared species from different geographical areas.

Site Selection

For the expanded geographical test of the $\delta^{15}N$ method to detect wastewater inputs to receiving waters, we used new data and published data for 31 ponds and estuaries in North and South America (Fig. 1). We collected new data from three estuaries (Mashpee River, Great Pond, and Green Pond, MA) and four freshwater ponds (Ashumet Pond, Coonamessett Pond, and Oyster Pond at southwestern Cape Cod and Miacomet Pond at Nantucket, MA). In addition, we collected data from Lamprey River and Oyster River (subestuaries of Great Bay, NH), Nick's Hole and Yent's Bayou (subestuaries of Apalachicola Bay, FL), and Piratininga and Itaipu (two portions of a coastal lagoon system in Brazil). We used previously collected or published data for Sage Lot Pond, Quashnet River, and Childs River, MA; Narragansett Bay, RI; Tijuana Estuary, San Dieguito Lagoon, and Elkhorn Slough, CA; South Slough, OR; and Padilla Bay, WA.

Macrophyte and Particulate Organic Matter $\delta^{15}N$ Measurements

For the sites in Cape Cod, Great Bay, and Apalachicola Bay in the USA, and Itaipu and Piratininga in Brazil, we collected emergent macrophytes, submergent macrophytes, and macroalgae from up to 10 locations within the freshwater ponds and estuaries (Table 1). Samples from all locations within a water body were combined into one composite sample per pond or estuary. Macrophyte tissues were dried at 60°C for 3 d, ground to a fine powder with a mortar and pestle, and stored in scintillation vials in a dessicator until analysis. The POM was collected in 2-L bottles from three locations around each site, filtered onto ashed glass fiber filters, dried at 60°C for 3 d, and stored in a scintillation vial in a dessicator until analysis. Nitrogen in macrophyte tissue and POM was then analyzed with a Finnigan Delta-S isotope-ratio mass spectrometer (Finnigan Corporation, San Jose, CA) coupled to a Heraeus element analyzer (Heraeus Instruments, Inc., South Plainfield, NJ).

Methods for collection and analysis of samples from Narragansett, RI, and the West Coast estuaries are found in Wigand et al. (2001), Kwak and Zedler (1997), and Fry et al. (2003). The Pacific Coast estuaries received significant freshwater inflow from their watersheds only during certain times of the year (Fry et al., 2003). Since we were most interested in the relationship of macrophyte and POM $\delta^{15}N$ to actual inputs of land-derived N rather than to N recirculated within the systems, we used only the samples collected at the time of lowest water-column salinity, which corresponded to recent freshwater inflow.

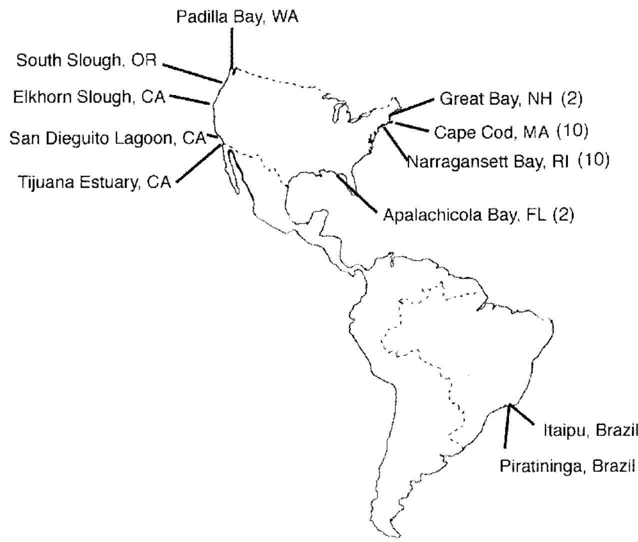

Fig. 1. Map of North and South America showing locations used in this study. Subestuaries and freshwater ponds of Cape Cod, Great Bay, Narragansett Bay, and Apalachicola Bay are given in Table 1. The number in parentheses indicates number of estuaries or ponds used for each area. If no number is provided, only one estuary was used for that location.

Calculation of Relative Wastewater Load

To determine the contribution of wastewater, fertilizer use, and atmospheric deposition to the total N load to the Cape Cod, Nantucket, and Narragansett Bay sites, we used a nitrogen-loading model (NLM; Valiela et al., 1997, 2000). To apply NLM, we first identified watershed boundaries using water table contours from U.S. Geological Survey maps (Savoie, 1995). We then compiled land uses for each watershed and subwatershed from aerial photos and geographic information system (GIS) software. The land uses were then entered into the NLM to calculate N loads from wastewater, fertilizer use, and atmospheric deposition (Table 1).

Water-Column Dissolved Inorganic Nitrogen Measurements

To compare $\delta^{15}N$ of macrophytes to ambient concentrations of dissolved inorganic N, we collected water from freshwater ponds and estuaries in Cape Cod, Great Bay, and Apalachicola Bay, and measured NO_3 and NH_4. We measured NH_4 concentrations colorimetrically by the phenol/hypochlorite method (Strickland and Parsons, 1972) or fluorometrically (Holmes et al., 1999). Nitrate concentrations were measured colorimetrically after cadmium reduction to NO_2 with either a manual method (Jones, 1984) or with an autoanalyzer (Lachat Instruments, Milwaukee, WI). The values arrived at by this method are actually $NO_3 + NO_2$, but because NO_2 concentrations are typically an order of magnitude lower than NO_3, we refer to this value as the NO_3 concentration. For the Brazil lagoons,

Table 1. Sites selected, macrophyte $\delta^{15}N$, particulate organic matter (POM) $\delta^{15}N$, water-column dissolved inorganic nitrogen (DIN) concentrations, and relative contribution of wastewater to modeled land-derived N load for freshwater ponds and estuaries in this study.

Site		Macrophyte $\delta^{15}N$	POM $\delta^{15}N$	Water-column DIN	Waste water as a percentage of total N load	Source
		‰	‰	μM	%	
Cape Cod, MA						
Mashpee River	*Enteromorpha* spp.	7.7	7.7	12.6	44	Cole (unpublished data, 2002)
	Gracilaria tikvahiae McLachlan	7.6				
	Spartina alterniflora Loisel	6.8				
Great Pond	*Enteromorpha* spp.	9.9	7.6	NA	66	Cole (unpublished data, 2002)
	Gracilaria tikvahiae	8.3				
	Spartina alterniflora	7.7				
Green Pond	*Enteromorpha* spp.	7.3	7.2	4.6	54	Cole (unpublished data, 2002)
	Gracilaria tikvahiae	8.5				
	Spartina alterniflora	8.1				
Ashumet Pond	*Hypnum* spp.	11.7	10.8	3.5	80	Cole (unpublished data, 2002)
	Potomogeton spp.	13.8				
	Callitriche palustris L.	6.4				
	Eleocharis spp.	7.4				
	Gratiola lutea Raf.	9.5				
	Ludwigia spp.	12.3				
	Elatine americana (Pursh) Arn.	11.3				
Coonamessett Pond	*Callitriche palustris*	4.7	7.2	NA	17	Cole (unpublished data, 2002)
	Elatine americana	6.8				
	Eleocharis spp.	5.3				
	Eriocaulon spp.	5.6				
	Gratiola lutea	4.2				
	Polygonum spp.	0.5				
Childs River	*Enteromorpha* spp.	8.2	5.7	3.5	65	McClelland et al. (1997), McClelland and Valiela (1998)
	Gracilaria tikvahiae	7.6				
	Spartina alterniflora	7.6				
Quashnet River	*Enteromorpha* spp.	6.6	4.7	1.8	30	McClelland et al. (1997), McClelland and Valiela (1998)
	Gracilaria tikvahiae	5.9				
	Spartina alterniflora	6.0				
Sage Lot Pond	*Enteromorpha* spp.	4.9	4.2	1.9	5	McClelland et al. (1997), McClelland and Valiela (1998)
	Gracilaria tikvahiae	5.1				
	Spartina alterniflora	4.4				
Oyster Pond	*Lemna* spp.	6.9	NA	5.2	71	this study
	Vaucheria spp.	10.3				
	Elatine americana	7.5				
	Eleocharis spp.	8.3				
	Myriophillum spp.	5.7				
	Najas spp.	6.4				
	Potomageton spp.	7.7				
Nantucket Island, MA						
Miacomet Pond	*Ceratophyllum* spp.	8.1	3.9	NA	27	Cole (unpublished data, 2002)
	Callitriche palustris	7.3				
	Elatine americana	6.3				
	Eriocaulon spp.	6.2				
	Najas spp.	6.2				
	Potomogeton perfoliatus L.	5.5				
	Ruppia maritima L.	2.9				
	Vallisneria americana Michx.	5.0				
	Typha latifolia L.	5.6				
Great Bay, NH						this study
Lamprey River	*Agardhiella tenera* Kraft & Wynne	10.3	8.1	5.2	NA	
	Ulvas spp.	9.7				
	Enteromorpha spp.	7.1				
	Zostera marina L.	6.4				
Oyster River	*Ulva* spp.	9.5	8.7	5.2	NA	
	Polysiphonia spp.	10.2				
	Ascophyllum spp.	9.4				
Apalachicola Bay, FL						this study
Nick's Hole	*Halodule wrightii* Asch.	3.7	7.2	0.3	NA	
Yent's Bayou			5.8	1.5	NA	
Narragansett Bay, RI						Wigand et al. (2001), McKinney (unpublished data, 2001)
Apponaug Cove	*Spartina alterniflora*	9.6	7.6	NA	75	
Bissel Cove	*Spartina alterniflora*	8.7		NA	63	
Brush Neck Cove	*Spartina alterniflora*	9.0	6.8	NA	82	
Donavan	*Spartina alterniflora*	7.4	5.2	NA	30	
Fogland	*Spartina alterniflora*	7.7	7.9	NA	25	
Foxhill Pond	*Spartina alterniflora*	5.7	6.6	NA	2	
Jenny Pond	*Spartina alterniflora*	6.3		NA	37	
Old Mill Creek	*Spartina alterniflora*	11.3	2.1	NA	79	
Passeonquis Cove	*Spartina alterniflora*	9.7		NA	82	
Watchemoket Cove	*Spartina alterniflora*	7.8	3.2	NA	70	
Tijuana Estuary, CA	*Ulva* spp.	11.5	6.0	188.1	NA	Kwak and Zedler (1997)

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Table 1. Continued.

Site		Macrophyte δ15N	POM δ15N	Water-column DIN	Waste water as a percentage of total N load	Source
		‰	‰	μ*M*	%	
Tijuana Estuary, CA	*Enteromorpha* spp.	11.9				
	Gracilaria spp.	11.4				
	Spartina foliosa Trin.	10.3	10.9			Fry et al. (2001)
San Dieguito Lagoon, CA	*Enteromorpha* spp.	11.4	9.4	NA	NA	Kwak and Zedler (1997)
	Gracilaria spp.	11.3				
Elkhorn Slough, CA	*Ulva* spp.	12.5	9.5	219.8	NA	Fry et al. (2001)
South Slough, OR	*Enteromorpha* spp.	6.5	4.3	56.7	NA	Fry et al. (2001)
Padilla Bay, WA	*Ulva* spp.	8.9	4.0	60.7	NA	Fry et al. (2001)
Brazil						
Itaipu Lagoon	*Laguncularia racemosa* (L.) C.F. Gaertn.	5.5	6.5	10.0	NA	this study
	Rhizophora mangle L.	1.5				Souza and Wasserman (1997)
	Avicennia germinans L.	8.0				
Piratininga Lagoon	*Enteromorpha* spp.	7.5	8.5	20.0	NA	this study
	Chara spp.	9.0				Souza and Wasserman (1997)
	Laguncularia racemosa	9.5				

and the Pacific Coast estuaries, we obtained DIN concentrations from Souza and Wasserman (1997) and Fry et al. (2001).

Statistics

To test whether the δ15N values of the macroalgae and plants related differently to wastewater N, we first used an analysis of variance (ANOVA; Statview 5.0.1, SAS, Cary, NC), to test whether the slopes of the two regression lines were significantly different (high *F* value, low *p* value). If they were not significantly different, we used an analysis of covariance (ANCOVA; Statview 5.0.1) with N load, wastewater, and DIN concentration as covariates to test if the *y*-intercepts of each regression line were significantly different (high *F* value, low *p* value).

Macrophyte δ15N Signatures as Indicators of Percentage Wastewater Inputs

We first regressed macrophyte δ15N and wastewater using macrophyte δ15N data from both Cape Cod and Narragansett Bay, RI. We then used that relationship to estimate the relative contribution of N by wastewater in estuaries where N loads were not available. For this part of the study, we used measurements of macrophyte δ15N from Great Bay, Nick's Hole, Itaipu Lagoon, and Piratininga Lagoon, as well as published data for Tijuana Estuary, San Dieguito Lagoon, Padilla Bay, South Slough, and Elkhorn Slough, and POM δ15N data for Yent's Bayou. To check on the plausibility of the prediction, we compiled whatever information was available on the land use and intensity of urbanization of their watersheds, to compare with the magnitude of calculated load.

RESULTS AND DISCUSSION

Macrophyte δ15N and Relative Wastewater Load and Water-Column Dissolved Inorganic Nitrogen

Regressions were developed to assess how all macrophytes responded to relative wastewater load and to water-column DIN concentrations. We then separated the macrophytes into macroalgae vs. plants and again assessed their relationship to relative wastewater load and to water-column DIN concentrations. Finally, we separated the macrophytes based on geographic location and again developed regressions to assess their relationship to relative wastewater load and to water-column DIN concentrations.

A variety of macroalgae and plants were found in all water bodies sampled (Table 1). The range of δ15N values was 0.5 to 13.8‰, a sufficiently large range for our test to be broadly applicable. The δ15N values of macrophytes increased as wastewater, as percentage of the total N load, increased (Fig. 2a and Table 2). This significant relationship is explained by the fact that wastewater δ15N values in ground-water-fed systems are heavier than fertilizer or atmospheric/soil N sources (Pabich et al., 2004). This analysis included many different species in freshwater ponds and estuaries across several geographic regions. Below, we break down this relationship into macroalgae vs. vascular plants, and into different geographic regions. We do not separate freshwater and estuarine species or rooted vs. nonrooted species since

Table 2. Regression statistics for linear regression between macrophyte δ15N and wastewater as a percentage of N load for Cape Cod and Rhode Island separately and combined. Data for Cape Cod is from Cole (unpublished data, 2002). Data for Rhode Island is from Wigand et al. (2001) and McKinney (unpublished data, 2001).

Site	Taxon	Regression equation	df	R^2	F_{reg}†	*p* value
	all macrophytes	$y = 0.07x + 4.15$	56	0.54	63.7	<0.001
	macroalgae	$y = 0.06x + 4.29$	5	0.91	41.4	<0.01
	plant	$y = 0.07x + 4.1$	44	0.52	46.0	<0.001
Cape Cod	*Spartina alterniflora*	$y = 0.07x + 4.55$	5	0.85	22.1	<0.01
Rhode Island	*Spartina alterniflora*	$y = 0.05x + 5.63$	9	0.71	19.4	<0.05

† F_{reg}, *F* regression.

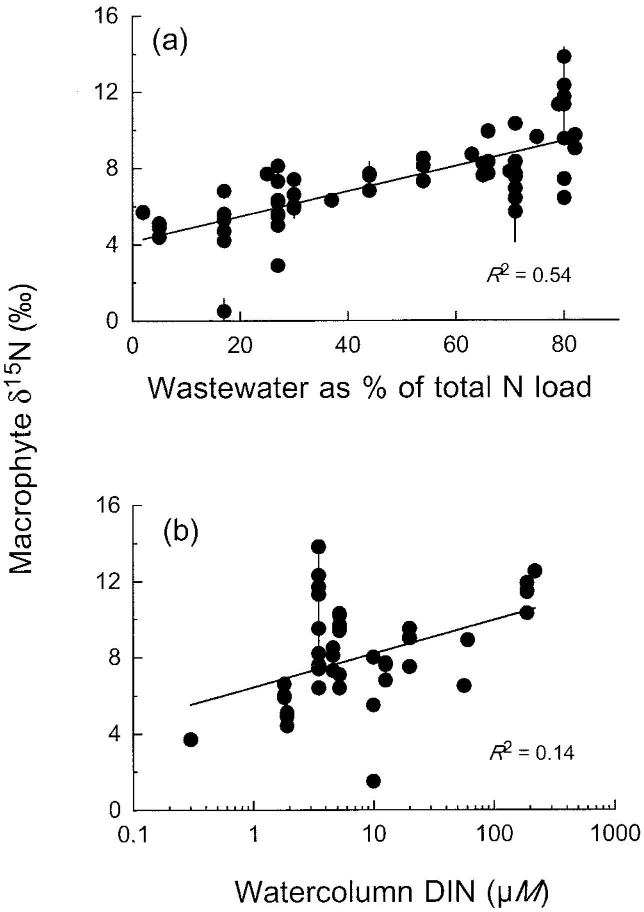

Fig. 2. (*a*) **Wastewater as a percentage of total N load modeled with the N-loading model of Valiela et al. (1997, 2000) vs. δ^{15}N of all species from all ponds and estuaries of study.** (*b*) **Water-column dissolved inorganic nitrogen (DIN) concentrations vs. δ^{15}N of all species from all ponds and estuaries of study. δ^{15}N values are means of all sampling dates, and error bars represent standard error.**

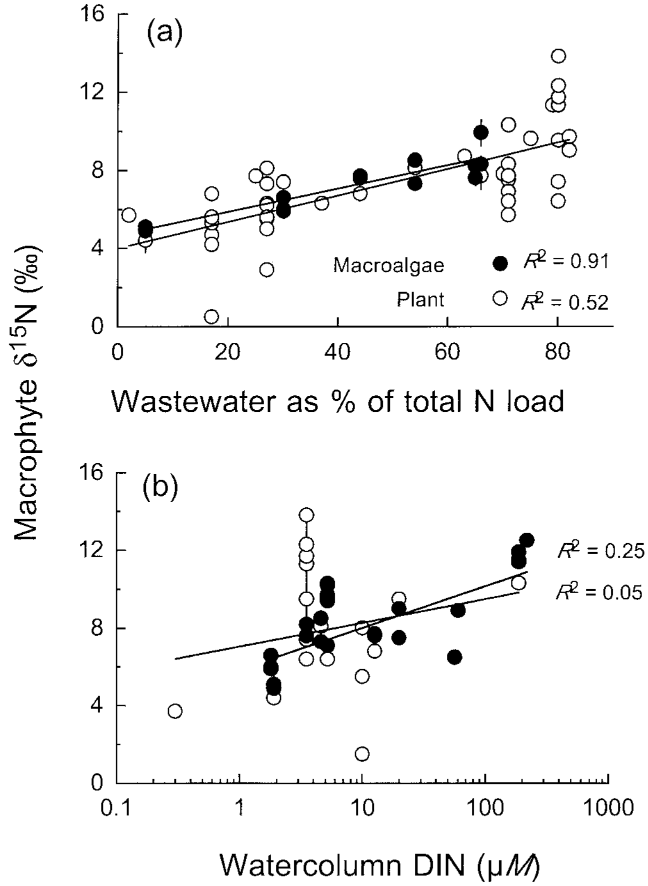

Fig. 3. (*a*) **Wastewater as a percentage of total N load as calculated with the N-loading model of Valiela et al. (1997, 2000) vs. δ^{15}N of macrophytes used in this study divided into groupings of macroalgae and plants.** (*b*) **Mean annual water-column dissolved inorganic nitrogen (DIN) concentrations vs. δ^{15}N of macrophytes used in study divided into groupings of macroalgae and plants.**

Cole (unpublished data, 2002) showed no difference in response of δ^{15}N values between these different groups of macrophyte species at Cape Cod.

Macrophyte δ^{15}N increased significantly as DIN in water from the estuaries where the collected producers increased (Fig. 2b and Table 3). In this case, the scatter of points was larger than for wastewater N. Nonetheless, on aggregate, N isotopic signatures did reflect ambient DIN concentrations in the estuaries. Lake et al. (2001) found a similar logarithmic relationship between consumers and water-column DIN concentrations in freshwater ponds. The δ^{15}N of producers can be affected by the concentration of DIN in the water column, regardless of its source. Higher water-column concentrations lead to high rates of N isotopic fractionation when producers assimilate DIN (Wada and Hattori, 1978; Fogel and Cifuentes, 1993), but this fractionation leads to lighter producer isotopic values. Thus, the increase of producer δ^{15}N values with DIN concentrations is less marked at higher concentrations (Cabana and Rasmussen, 1996; Lake et al., 2001). Macrophyte δ^{15}N values, then, are sensitive mainly to low DIN concentrations. Although there is a significant relationship between macrophyte δ^{15}N values and water-column DIN concentrations, the overall poor R^2 of 0.14 suggests that the relationship may not be a particularly useful predictor.

Table 3. Regression statistics for linear regression between macrophyte δ^{15}N and water-column DIN concentrations. Data and sources listed in Table 1.

Taxon	Regression equation	df	R^2	F_{reg}†	*p* value
All macrophytes	$y = 1.75 \log x + 6.44$	42	0.14	7.0	<0.05
Macroalgae	$y = 2.14 \log x + 5.87$	24	0.25	7.5	<0.05
Plant	$y = 1.83 \log x + 6.72$	18	0.05	0.8	>0.05

† F_{reg}, *F* regression.

Both macroalgae and plant δ^{15}N values significantly increased as wastewater N increased as a portion of the total N load (Fig. 3a and Table 2). There was no statistical difference by ANCOVA between the slopes and intercepts of the two regressions. Plants had a larger range of δ^{15}N values (0.5–13.8‰) than macroalgae (4.9–9.9‰), but this difference was likely due to the sampling of many more plant species than macroalgae. One might expect differing responses of macroalgae and plants to increases in wastewater N because of differences in N uptake rates in preference for NO_3 or NH_4 in internal N cycling rates and in N sources. Plants have access to porewater N and water-column N, while macroalgae

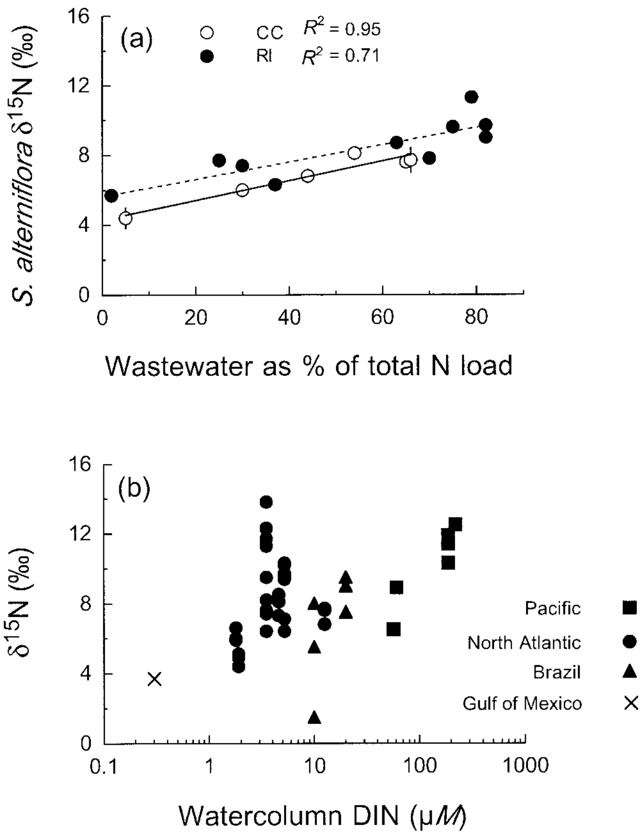

Fig. 4. **(*a*) Wastewater as a percentage of total N load as calculated with the N-loading model of Valiela et al. (1997, 2000) vs. δ^{15}N of *Spartina alterniflora* for two geographic groups of ponds and estuaries: Cape Cod (CC) and Rhode Island (RI). (*b*) Mean annual water-column dissolved inorganic nitrogen (DIN) concentrations vs. δ^{15}N of macrophytes grouped in four geographic regions. δ^{15}N values are means of all sampling dates, and error bars represent standard error.**

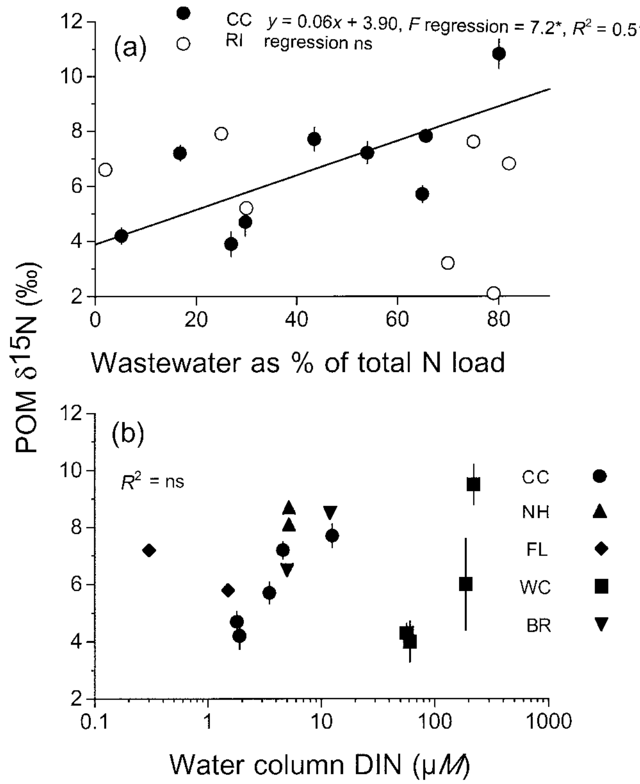

Fig. 5. **(*a*) Wastewater as a percentage of total N load as calculated with the N-loading model of Valiela et al. (1997, 2000) vs. particulate organic matter (POM) δ^{15}N for six sites of present study, three sites of McClelland et al. (1997) and McClelland and Valiela (1998), and six Narragansett Bay estuaries. (*b*) Mean annual water-column dissolved inorganic nitrogen (DIN) concentrations vs. POM δ^{15}N for sites in Cape Cod (CC), New Hampshire (NH), Florida (FL), U.S. West Coast (WC), and Brazil (BR). δ^{15}N values are means of all sampling dates, and error bars represent standard error.**

can take up N only from the water column. Despite these complex factors, there was no difference in slope (F = 0.2, not significant) or y-intercept (F = 0.39, not significant) in the two responses of macroalgae and plants to increases in wastewater load. In summary, although both relationships are good, the macroalgae relationship is tighter. This suggests that when macrophytes are separated into vascular and nonvascular groups, nonvascular macroalgal δ^{15}N values would be a better predictor of wastewater N.

Macroalgal δ^{15}N values increased significantly with water-column DIN concentrations, while plant δ^{15}N values did not (Fig. 3b and Table 3). The two regressions had similar slopes. Macroalgae may respond significantly because they only have access to water-column DIN, but plants have access to ground water and porewater N and therefore may not be coupled to the water-column N. In summary, macroalgae are better indicators of the water-column DIN concentrations than plants.

In different estuaries with a range of watershed land uses, *S. alterniflora* δ^{15}N values responded similarly to wastewater inputs (Fig. 4, statistics in Table 2). As wastewater input increased, *S. alterniflora* δ^{15}N values for both geographic areas were significantly enriched in ^{15}N. Lake et al. (2001) found a similar response of freshwater pond sediment and consumer δ^{15}N relative to the fraction of residential land in pond watersheds. Although the relationships for the two geographic regions (Cape Cod and Rhode Island) had the same slope, the regressions were offset by a small amount, 1.3‰. This minor difference is likely due to differences in estuary processing between the two regions. Regardless, the regressions of one plant species, *S. alterniflora*, to wastewater inputs calculated for two different geographic regions were not different (F = 0.14, not significant).

To further analyze the geographical differences we grouped macrophyte δ^{15}N values into different geographic regions, and correlated the δ^{15}N signatures vs. the water-column DIN concentrations (Fig. 4b). The Pacific estuaries had high DIN concentrations, but macrophyte δ^{15}N values spanned nearly the same range as those in the northern Atlantic and Brazilian estuaries. These Pacific estuaries have limited inflow of freshwater, so we used data only from seasons with freshwater inflow to best capture inputs from land. Fry et al. (2003) attribute high concentrations during the high flow season to direct agricultural runoff and sewage effluent. The Brazilian lagoon water-column DIN concentration values fell between those of the Pacific and north Atlantic regions. The two Gulf of Mexico estuaries had low δ^{15}N values and low DIN concentrations. Both estuaries are fairly pristine with relatively undeveloped watersheds.

Particulate Organic Matter $\delta^{15}N$ and Relative Wastewater Load and Dissolved Inorganic Nitrogen Concentrations

The POM $\delta^{15}N$ values were significantly related, although with large scatter, to percentage wastewater in Cape Cod water bodies, but the relationship was not significant when Narragansett Bay data were included (Fig. 5a). The POM $\delta^{15}N$ values were not significantly related to water-column DIN concentrations (Fig. 5b). This is contrary to findings of Cole (unpublished data, 2002), where data from a more geographically restricted area showed that $\delta^{15}N$ values were related logarithmically to water-column DIN concentrations in Cape Cod water bodies.

The $\delta^{15}N$ values of POM seem to be less reliable indicators of land-derived N than those of macrophytes. In fact, changes in phytoplankton community structure across time may occur, and different species may fractionate N to different degrees. For example, cyanobacteria blooms induce lower $\delta^{15}N$ values in water-column DIN (Peterson and Fry, 1987; Fogel and Cifuentes, 1993; Kendall, 1998). The POM includes not only phytoplankton, but also particles from sediments, aggregates of DOM, macrophytes, or terrestrial origin. New methods have recently been developed to assess $\delta^{15}N$ values of phytoplankton chlorophyll (Sachs et al., 1999; Sachs and Repeta, 2000). The new methods were developed too recently to be of use to this study, but future studies could incorporate them to better assess the relationship between phytoplankton $\delta^{15}N$ values and land-derived N.

Use of $\delta^{15}N$ of Macrophytes to Estimate Percentage Wastewater Inputs

The relationship of $\delta^{15}N$ of macrophytes to percentage wastewater, defined in Fig. 2, was sufficiently good that we ventured to use that relationship to estimate the percentage wastewater N for estuaries for the estuaries where data were unavailable (Lamprey River and Oyster River, NH; Nick's Hole and Yent's Bayou, FL; Piratininga Lagoon and Itaipu Lagoon, Brazil; Tijuana Estuary, San Dieguito Lagoon, and Elkhorn Slough, CA; Padilla Bay, WA; and South Slough, OR). We could not directly verify these $\delta^{15}N$-based estimates to measurements, but we could compare the estimates of wastewater N to available qualitative information on watershed land use for each of these estuaries.

In general, the estimated wastewater percentages matched what is known of watershed land uses for those estuaries (Table 4). In all cases, estimated wastewater percentages were high (62–114%) where there were wastewater inputs from septic systems, wastewater treatment plant outfalls, cattle grazing, or direct releases of raw sewage. Where the watershed had little development, the estimated wastewater percentages were low (0–32%). These comparisons suggest that the $\delta^{15}N$ method might be useful where N-load estimates might not be available.

Estimates of the percentage of wastewater N based on macrophyte $\delta^{15}N$ may be inaccurate if other N sources have a strong effect on $\delta^{15}N$ values of macrophytes. The wastewater estimates may be too high if N sources with heavy $\delta^{15}N$ signatures such as coastal upwelling and regeneration contribute a significant proportion of the N available to macrophytes. Fry et al. (2003) suggest that agricultural runoff in regions with high rates of soil denitrification may have $\delta^{15}N$ values close to that of

Table 4. The $\delta^{15}N$ of macrophytes and estimated percentage of wastewater N inputs for 12 estuaries.

Estuary	$\delta^{15}N$†	Estimated waste water	Ranking‡	Land use	Source
	‰	%			
Itaipu Lagoon, Brazil	3.9§	0	L	watershed largely natural vegetation with some houses	Souza and Wasserman (1997)
Nick's Hole, FL	5.5	22	L	relatively pristine, some in flow from Apalachicola Bay containing water with septic or sewage effluent	
Yent's Bayou, FL	5.8	25	L	relatively pristine, some in flow from Apalachicola Bay containing water with by septic or sewage effluent	
South Slough, OR	6.5§	34	L	watershed contains only modest residential development and agriculture	Fry et al. (2001) Roegner and Shanks (2001)
Lamprey River, NH	8.6	62	I	wastewater treatment plant outfalls, septic systems	Jones and Langan (1996)
Piratininga Lagoon, Brazil	8.6§	63	I	bordering villages release untreated sewage	Souza and Wasserman (1997)
Padilla Bay, WA	8.9§	66	I	watershed with some residential development, but mainly agricultural	Bernhard and Peele (1997) Cassidy and McKeen (1999) Fry et al. (2001)
Oyster River, NH	9.3	72	H	wastewater treatment plant outfalls, septic systems	Jones and Langan (1996)
San Dieguito Lagoon, CA	10.7#	90	H	highly developed watershed, horse track and stables near estuary	Kwak and Zedler (1997) Greenwald and Hurlbert (1993)
Tijuana Estuary, CA	11.1#, 12.1¶	96, 109	H	watershed highly developed, wastewater treatment plant outfall from San Diego, CA, and untreated sewage from Tijuana, Mexico	Kwak and Zedler (1997) Fry et al. (2001)
Elkhorn Slough, CA	12.5¶	114	H	large percentage of watershed in cattle grazing and agriculture	Scharffenberger et al. (1999) Fry et al. (2001)

† This study unless otherwise noted.
‡ The ranking was based on land use. L = watershed largely undeveloped; I = some watershed development, either residential or agricultural; H = watershed highly developed.
§ Muto et al. (unpublished data, 2000).
¶ Fry et al. (2001).
# Kwak and Zedler (1997).

wastewater. The wastewater percentage might be underestimated if sources of N with light $\delta^{15}N$ signatures, such as atmospheric deposition, N_2 fixation, and nitrification (Kendall, 1998), are available in the water column for producer uptake. In spite of these caveats, the method using macrophyte $\delta^{15}N$ to identify relative inputs of land-derived wastewater worked well in the sites of this study.

CONCLUSIONS

Macrophyte $\delta^{15}N$ was a reliable indicator of relative wastewater load to receiving waters and, to a lesser extent, of water-column DIN across a wide geographic range. The stable isotope method for detecting wastewater works equally well for macroalgae and plants across different geographic regions, while macroalgal $\delta^{15}N$ values were a better tracer of water-column DIN concentrations than vascular plants. This stable isotopic method can detect wastewater and DIN at low and high levels, making it useful for identifying incipient eutrophication before water bodies begin to show effects of eutrophication. The results for this research provide an inexpensive and simple tool to assess effects of watershed urbanization on coastal water bodies. For example, the derived relationship between *Spartina* $\delta^{15}N$ and percentage of wastewater is currently being used in the assessment of the N source for a large noxious macroalgal bloom in a small Rhode Island estuary with a relatively undeveloped watershed. The results of this assessment will help determine a restoration plan for the estuary. This is just one example of the possible uses of this stable isotope method.

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